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The Search for the Origin of Life: Some Remarks About the Emergence of Biological Structures

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1. Introduction

In the chapter "Religious Opinions" of his *Autobiography*, Darwin sustains that, after the law of natural selection has been discovered, the old argument about a "design" of nature as written by Paley falls apart, the same argument which he believed to be decisive in the past. So, in accordance with Darwin, we can no longer argue, for example, that the perfect hinge of a bivalve shell must have been conceived by an intelligent being, like the hinge of a door by man:

A design regulating the variability of living beings and the action of natural selection is not more evident than a design preparing the course of the wind. All that exists in nature is the result of fixed laws. (Darwin, 1958, p. 69).

Darwin's theses, as it is already known, have gradually influenced the entire progress of science. However it is necessary to underline the fact that, at the moment, nobody knows with certainty how life has begun. The question concerning the origins pervades, since the dawn of history, the most elevated exercise of human reason and it represents, for some aspects, a question that appears to interest the entire horizon of reason and, with it, the same dimension that is proper to the category of possibility. In this sense, from the dawn of civilization to the present, every human being and especially every scientist has measured himself within the horizon of that question, laying special emphasis, within his/her own intellectual limits, on offering a satisfying answer to that question itself. The apparent inexhaustibly connected to the question about the origin of life appears to exalt the Dantesque-like contradiction between the impetus towards the necessity for an objective knowledge of the nature of life and the limitation, instead, of the measures established by mankind in order to give a scientific explanation of said question. As a matter of fact, we might never be able to re-build the real historical sequence of the events which allowed the first molecular systems to evolve and reproduce more than 3 billion years ago. Still, if on the one hand the historical course is bound to stay, maybe forever, a mystery, on the other hand it is possible to develop structured theories and experiments to demonstrate, in an almost realistic way, how life may have first crystallized and later spread to the entire globe (Di Bernardo, 2007a, 2007b). In this sense, it is not entirely surprising then that the progress in the understanding of possible paths towards the origins of life extends across several scales

from the molecular to the cosmos. The individualization of such paths has resulted in alternative conceptions which have been examined by the researchers. Theses conceptions have led, also through the graft of molecular biology on Darwin's ancient evolutionary ideas to a standard theory; a theory which could be revised and enlarged, as it seems to happen nowadays, to merge with the original complexity theory elaborated first by Kauffman (1993, 1995). Complexity theory in recent years, has come to be appreciated by the scientific community as the most plausible outline capable of giving an answer to the problem of how life emerged from simpler molecular components. The great mystery of biology, according to the American biochemist, lies exactly in the fact that life has emerged and the order we observe has appeared: a theory of emergence should give account of the creation of the astounding order we see from our window as the natural expression of some underlying law. This would tell us if we are "at home in the universe", if here we are "expected" rather than being present against all probability (Kauffman, 1995).

2. Tracing the origins

In the volume *At Home in the Universe: the Search of the Laws of Self-Organization and Complexity,* Kauffman (1995) examines seven hypotheses concerning the attractive question about the origin of life.

The first one dates back to the first half of the twentieth century when scholars focused their attention mostly on the nature of the earth's primitive atmosphere which had given origin to the chemical molecules of life. Much evidence was accumulated to show how the first atmosphere was rich in molecular species such as hydrogen, methane and carbon dioxide, whereas oxygen was almost completely absent (today there are some doubts concerning such evidence). Very soon, the hypothesis that simple organic molecules, which are present in the atmosphere slowly dissolved in recently formed oceans to create a prebiotic broth from which life would have spontaneously emerged was postulated. Nevertheless, Kauffman highlights the main limit of this theory; i.e. the fact that the broth would have been extremely diluted. The quantity of chemicals reactions depends on how quickly the molecular species which reacts are able to meet- and this depends on how high their concentration is. If the concentration of each one is low, the possibility that they collide is really small (Kauffman, 1995).

The second hypothesis is attributed to A. Oparin, a Russian biochemist who proposed a plausible way of dealing with the problem of the diluted broth. When Glycerine is mixed with other molecules it forms some gelatinous structures called coacervates, structures similar to primitive cells. The coacervate, in fact, is able to concentrate in itself organic molecules and exchange them through its external surface. If these microscopic compounds had developed themselves in the primordial broth, they might have concentrated the chemical substances suitable for the formation of metabolisms. Yet, if Oparin laid the foundations to understand how protocells may have formed, he left the question concerning the origin of their contents, i.e. the organic molecules responsible for metabolic activity, completely unsolved. Together with the simple molecules there are several polymers, long molecular chains made almost by the same basic elements. The proteins out of which muscles are made, the enzymes and the structure of the cells, are formed by chains of twenty types of amino acids. The DNA and RNA are composed by chains of four basic elements (the nucleotides): adenine, cytosine, guanine and thymine in the DNA, with uracil

instead of thymine in the RNA. Without these molecules, the real material of life, including Oparin's coacervates would have been nothing other than empty shells (Kauffman, 1995).

But where could these basic elements have come from? The third hypothesis attempts to answer this question. In 1952 S. Miller, a young pupil of H. Urely, conducted the first experiment of pre-biotic chemistry: he filled a container with some gas (methane, carbon dioxide and others) which were believed to be in the atmosphere of primitive Earth. He then subjected the container to a "rain" of sparks to simulate lighting as a source of energy and he awaited in the hope of obtaining some proofs of molecular creativity. After a few days he observed that clots of brown matter stuck to the sides and to the bottom of the container. After analyzing them he found out that the material contained a large variety of amino acids. Similar experiments have demonstrated that it is possible, although with extreme difficulty, to form nucleotides of both DNA and RNA, fat molecules and, through them, it is the structural material constituting the cellular membranes. Many other little molecular components of the organisms have been synthesized in the abiogenic way (Kauffman, 1995). However, Robert Shapiro (1986) in the *Origins, a Skeptic's Guide to the Creation of Life on Earth*, underlines the essential problems of this hypothesis. He, in fact, reaffirms that even if scientists would be able to demonstrate how it is possible to synthetize the various ingredients of life, it is not easy at all to fit all this into a single story. One can establish, for instance, that the molecule A can be formed by the molecules B and C with a very low performance in certain conditions; therefore, having demonstrated the feasibility of A, another group begins with an high concentration of molecules and demonstrates that by adding D we can obtain E (again with a small performance and in different conditions); then, still another group demonstrates that E, in high concentrations, can form F in totally different conditions. That being so, Kauffman, quoting Shapiro, writes:

But how, without supervision, did all the building blocks come together at high enough concentrations in one place and at one time to get a metabolism going? Too many scene changes in this theater […] with no stage manager. (Kauffman, 1995, p. 36).

The fourth hypothesis takes as its theoretical reference-point the discovery of the genetic molecular structure by Watson and Crick in 1953. The discovery of the DNA double helix is, in fact, the final event which has prompted a renewed interest, within the scientific community, in the origin of life. It is hard not to be amazed by how the DNA double-helical structure immediately suggests the way molecules replicate themselves. Each filament specifies the sequence of nucleotides in the complementary filament thanks to the precise pairing of A-T and C-G, respectively. If DNA is a double helix in which each filament is complementary to the other, it means that the DNA double helix might be a molecule able to replicate itself in a spontaneous way. DNA, in brief, becomes the candidate for the first living molecule. It is considered to be the most important molecule in current life, the bearer of the genetic program responsible for the creation of the organism out of a fertilized egg. This magic molecule might have been the first to self-reproduce at the dawn of life. The molecule would have multiplied, finally running into the recipe for making proteins and creating its structure, and for speeding up its reactions by catalyzing them.

However, M. Meselson and F. Stahl (1958) highlighted the limits of the theory by demonstrating that the chromosomal DNA, inside the cell, even if it replicates as suggested by its structure, needs the help of an ensemble of protein enzymes. In other words, the two biochemists found out that DNA alone does not self-replicate. This being the case then, the

scholars who were looking for the first living molecule had to look towards RNA or ribonucleic acid which has a leading role in the functioning of the cell. RNA can exist as a single filament or as a double helix and, as it happens in the DNA, the two filaments of the RNA double helix are complementary. In the cell the information necessary to make a protein is copied by DNA in a filament called messenger RNA which is later transferred towards the ribosomes, structures where, with the help of transfer RNA, proteins are produced. Very soon, in the eyes of the scientific community, given the complementarity of the two RNA filaments, it seemed possible that such a molecule could be able to self-replicate without the help of protein enzymes.

The fifth hypothesis thus is called "RNA world" hypothesis and it suggests that life began with a proliferation of RNA molecules (defined by the scholars as "naked genes"). The basic idea of this hypothesis is quite simple: a high concentration of a single filament specified sequence is put into a cylinder (it can be a decanucleotide CCCCCCCCCC) with the subsequent addition of some highly concentrated free nucleotides G; now each G should pair up with one of the nucleotides C of the decanucleotide, - as it is stated by the principle of molecular complementarity postulated by Watson and Crick -, so as to form a group of aligned G adjacent with each other. It is sufficient that the ten nucleotides of G combine with one another through appropriate bonds. Thus we would have the formation of a poly-G decamer. At this point the two filaments (poly-G and poly-C) only need to separate and let the poly C decamer free to align other ten monomers G thus creating another poly G decamer. In the end, the new poly G decamer, in order to obtain a system of molecules which is able to self-replicate, should be able to align any free monomer C which has been added in the cylinder to form a poly C decamer. If what has just been said would actually occur without the adding of any enzyme, we would be in the presence of a naked double-stranded RNA molecule able to self-replicate.

The idea is beautiful and attractive. Anyhow, almost without exceptions the experiment failed. The reasons why it does not work are extremely instructive. Firstly, each of the four nucleotides has a chemical personality of its own which tends to make the experiment fail. Thus a single filament poly-G has the tendency to roll up on its own in order to make two nucleotides G link together. The result is a tangled hank unable of acting as a stamp to self-replicate. Even if the process of copying would not be interrupted by the tangling of the guanine, the naked RNA molecules could be the victims of what we call an error catastrophe: during the copying of a filament into another one, the exchange of some bases- a G in place of a C- would alter the genetic message. In the cells these errors are kept at minimums levels by controlling enzymes assuring the fidelity of the copying process. But without the enzymes which prevent the guanine from tangling, one can only think of the number of copying errors and other mistakes; these errors can result in a RNA message soon lose all meaning. And in a world of RNA in its pure form, where would the enzymes have come from? (Kauffman, 1995).

3. The standard model

The fourth and fifth hypotheses share the idea of mold replication. It is for this reason that, according to many scholars, they meet in what is defined as the standard theory of the origin of life: a hypothesis stating that life should be based on mold replication; i.e. a replication similar to the one we find in the DNA double helix or in the naked double-stranded RNA molecules.

Under some aspects then, in the aperiodic[1] sequence of bases of today it is possible to recognize the Schrodinger's aperiodic solid with its micro code. As imagined by Schrödinger, in fact, the sequence of the bases is arbitrary, therefore it is able to "tell" different things, to encode information (Schrödinger, 1944). The arbitrary sequence can be interpreted as the effective carrier of the genetic code: the triplets of bases are the codons and sixty-one, out of the sixty-four possible triplets of codons, encode the twenty standard amino acids, while the three remaining codons specify stop-signals to the translation. Moreover, the symmetry of the DNA molecule allows the arbitrariness of the bases to be coherent with the mechanism of mold replication. According to this model, the origin of life must have been based on some sort of double chained aperiodic solid. Well, as we have just mentioned, this idea has been proven wrong, nobody has been able to create the experimental conditions which allows a single DNA or RNA chain to align free nucleotides, one at the time, as complementary elements of a single chain; to catalyze the soldering of free nucleotides to form a second chain; to separate the two chains and enter a new replication cycle.

The limits of this model have already been emphasized since the second half of the 1960's by many scholars, among whom Monod undoubtedly stands out. The French biologist, in *Le hasard et la necessite: essai sur la philosophie naturelle de la biologie moderne*, wrote as follows:

One could think that the discovery of the universal mechanisms on which the essential properties of the living beings are based has allowed the resolution of the problem of the origins. As a matter of fact such discoveries, by presenting this question in a new light, a question which today is put in more precise terms, have made the said question more complex than what it seemed before. (Monod, 1970, p. 128).

In the process that must have led to the appearance of the first organisms, the French scientist defined a priori three phases: a) the formation on the earth of the essential chemical constituents of all living beings, precisely nucleotides and amino acids; b) the formation, starting from such substances, of the first macro molecules able to replicate; c) the evolution which has built, around these "replicative structures", a teleonomic apparatus able to lead to the primitive cell. According to the scholar, the first phase is accessible both from a theoretical and experimental point of view. Although we will never know the paths the pre-biotic evolution has really followed, the overall picture is quite clear: the conditions of the atmosphere and those of the earth's crust favoured the accumulation of certain simple compounds of carbon as for instance methane (there were also water and ammonia). Now, according to Monod, from such simple compounds, and in the presence of non-biological catalysts, we obtain quite easily several more complex substances among which we have amino acids and some precursors of nucleotides (azotic bases and sugars). It seems to be demonstrated that, on the earth, at a certain moment, some expanses of water were in the conditions of containing high concentrations of the essential constituents of the two classes of biological macro molecules, nucleic acids and proteins. In this primordial broth different

[1] In an attempt to explain the stability of the gene, Schrödinger begins by making some considerations on the quantum states of atoms and the mutagenic effects of ionized radiations. After an estimate of the gene's dimension in terms of number of atoms, the scientist shows that the only sufficiently stable way in which the atoms can be kept together is by forming a molecule (hypothesis already approached by Delbrück). He put forward the famous hypothesis according to which the gene is constituted by an "aperiodic crystal", i.e. a large-sized molecule with a non-repetitive structure, capable of (providing) a sufficient structural stability and a sufficient capability to contain informations (cfr. , Schrödinger, 1944, pp 100, 106-107).

macro molecules were able to form themselves through polymerization of their precursors; i.e. nucleotides and amino acids. In the laboratory we have obtained in fact, in plausible conditions, some polypeptides and polynucleotides with a general structure similar to that of modern macro molecules (Monod, 1970). The second point is about the formation of macro molecules which are able, in the same conditions of the primordial broth, to promote their own replication without the cooperation of a teleonomic apparatus. Such difficulty, according to the French biologist, has been surpassed by demonstrating that a poly nucleotide sequence can actually lead, through a spontaneous pairing, the formation of elements with complementary sequence. Obviously a similar mechanism was slightly efficient and subjected to countless errors, however, from the moment it went into action, according to Monod, the three fundamental processes of the evolution- replication, mutation, selection- started to operate giving a considerable advantage to the macro-molecules which, thanks to their sequential structure, were more suitable to spontaneously replicate .The third phase consists in the gradual appearance of those teleonomic systems which had to build an organism (a primitive cell) from and around the replicative structure:

Here we really once again meet a wall of resistance, since we do not have the smallest idea of what the structure of a primitive cell was. The simplest living system we are aware of, the bacterial cell, an extremely complex and effective small device, had already got to its actual state of perfection more than a thousand million years ago. The development of the metabolic system which, while the primordial broth was gradually being impoverished, had to learn how to mobilize the chemical potential and synthetize the cellular constituents, poses huge problems. The same difficulties apply for the appearance of the membrane equipped with selective permeability and without which the existence of vital cells would not be possible. Yet the most serious problem concerns the origin of the genetic code and its mechanism of translation. More properly, instead of addressing this matter as a problem, we should consider it an enigma. (Monod, 1970, p. 131).

The origin of the code, in Monod's opinion, constitutes that disturbing border of the unknown which the evolution shows to its extreme: the code does not make sense if not translated. The translation mechanism of the modern cell entails at least fifty macro molecular constituents, also encoded in the DNA. It means that the genetic code can only be translated by the same products of translation. This is the modern expression for the *omne vivum ex ovo*. But when and how did this ring close on itself? It is in this moment that Monod transforms the scientific study of life in an interrogation in fact there is still no answer to this question but only hypotheses.

4. Before the genetic code

Hence, given what has been demonstrated up to now, the sixth and seventh hypotheses which we are going to outline consist of two different attempts to exceed the biological aporia highlighted with great shrewdness by the French biologist. Towards the mid-eighties, T. R. Cech, together with his collaborators, found out that the same RNA molecules could act as enzymes and catalyze reactions; very soon, these RNA sequences were defined as ribozymes (Kelly et al., 1982; Atkins et al., 1993, 2011). When the DNA message (the instructions to encode a protein) is copied on a filament of messenger RNA, a certain quantity of information is ignored. The cells do not have only "revising enzymes" but also "editing enzymes". The regions of the sequence containing the genetic instructions (exons) must be separated by the noncoding DNA sequences (introns). Thus, some enzymes remove

the introns from the RNA and unify the exons. Subsequently, the sequence of exons is still processed in various ways; it is carried outside the nucleus and translated into a protein in the ribosome. Cech (Atkins et al., 1993) discovered, not without much stupor, that in some cases a protein enzyme is not necessary for the editing, because it is RNA itself which acts as an enzyme, so as to eliminate its own introns. The results amazed the community of molecular biologists. Today we know that a great variety of different ribozymes exists and they are able to catalyze different reactions by acting on themselves or on other RNA sequences. RNA molecules, in the absence of protein enzymes, are rather clumsy when they have to self-reproduce. But maybe a RNA ribozyme can act as an enzyme and catalyze the reproduction of RNA molecules: a similar ribozyme could act on itself to self-replicate. In both cases, a self-reproducing molecule, or a system of molecules would be at hand. Life would be on the right track (Kauffman, 1995). Yet there is a serious problem about the possibility of thinking of the ribozyme with polymerase activity as the original molecule of life. Even if such molecule had developed itself, in fact, could it have protected itself from a deterioration caused by mutations? Could it have evolved? According to Kauffman the answer to this question is probably a negative one. The problem is a form of error catastrophe, described for the first time by the chemist L. Orgel in the context of the genetic code. Let us imagine a ribozyme which is able to function as polymerase and copy every RNA molecule, itself included. This ribozyme, having a source of nucleotides at its disposal, would act as a replicating naked gene. Nevertheless any enzyme only accelerates the correct reaction among the possible alternative secondary reactions which could occur. Errors are inevitable. The self-reproducing ribozyme would necessarily come to reproduce also some mutant variations. But those mutant ribozymes are probably less effective than the normal ribozyme and so they have a high probability of committing errors more frequently. After some cycles, the system could produce an incontrollable spectrum of mutant variations. "Life would have vanished in a runaway error catastrophe" (Kauffman, 1995, p. 42).

Among all the problems related to the sixth hypothesis, the most original is without a doubt the one about the minimal complexity threshold of living creatures: all living organisms, according to Kauffman, seem to have a minimal complexity threshold below which life cannot exist. In nature the simplest free cells are called *pleuromona;* a simplified bacterial species infesting sheep lungs. Unlike viruses which are not living organisms[2], all cells have at least the basic molecular difference of pleuromona, which means they have at least a membrane, a DNA, a code, three hundred assorted genes, a transcription and translation device, a metabolism and a connection of energy fluxes towards and through the inside. Why, therefore, do free cells have an apparent minimal complexity? Kauffman thinks that a positive aspect of the naked gene theory is the simple origin of life, while its negative side is surely the fact that it does not articulate a satisfying answer to the question just posed. In brief, naked RNA, or the naked ribozyme with polymerase activity does not offer large

[2] The fundamental characteristic differentiating what is alive from what is not can be found in the self-construction principle. All living organisms, from single cells to multicellulars, can produce their own components through an autonomous process of organization: the internal dynamic of the living being itself is, in the last analysis, responsible for the organization of nature. This would be the reason why viruses, which need to invade the cell in order to reproduce, cannot be considered living organisms to all intents and purposes: they are incapable of self-construction and self-reproduction; as in the case of prions, i.e. the infectious agents of protein nature which are responsible for degenerative brain diseases like "mad cow disease" but they have no nucleic acids and thereby no genetic code.

bases to understand the minimal complexity observed in all living cells. Kauffman considers as a virtue of the theory of the origins the fact that it explains why the matter must reach a certain degree of complexity before life could emerge: "This threshold is not an accident of random variation and selection; I hold that it is inherent to the very nature of life" (Kauffman, 1995, p. 43). G. Wald (1954), in the *The Origin of Life*, wonders how it is possible that a group of molecules can assemble themselves in the exact way to form a living cell and goes on to affirm that, if many attempts are made, what is "inconceivably improbable" becomes "virtually certain". Hence, given so much time, according to this seventh hypothesis, the impossible becomes possible, the possible probable and lastly the probable virtually certain. Therefore, life, for Wald, would have emerged from a pure random combination modeled in stages by time. Such perspective however, is not so distant from Monod's vision who, concluded:

Modern science ignores any immanence. Destiny is written in the moment it is fulfilled and not before. Our destiny was not fulfilled before the appearance of the human species, the only species in the universe able to use a logic system of symbolic communication. Another unique event, which should, exactly for this reason, stops us from any form of anthropocentrism. If this event has really been unique, as the case of the appearance of life, this depends from the fact that, before it showed itself, its possibilities were almost null. The universe was not about to conceive life, nor the biosphere was about to conceive man, our number has come up on a roulette: why, therefore, should we not be aware of the exceptionality of our condition, like the one who has just won a thousand million? (Monod, 1970, pp. 133-134).

However much he wants to reduce all that happens to mere chance, Monod cannot refrain from asking himself a question given the exceptional nature of the phenomenon of self-consciousness. Yet R. Shapiro (1986), answers this question in a very different way: in fact, he calculates that, in the earth's life, $2{,}5 \times 10^{51}$ casual attempts to create life could have occurred. That is a very large number of attempts. Shapiro goes on with his research trying to calculate the possibility of obtaining, casually, something like *E. coli* (Shapiro, 1986). He then begins with the argument supported by two astronomers, F. Hoyle and N. Wickramasinghe, who, instead of calculating the probability of obtaining a whole bacterium, tried to calculate the possibility of obtaining a functioning enzyme (Hoyle & Wickramasinghe, 1981, 1993, 2000). Thus, if the amino acids are selected and structured in a casual way, what would be, then, the probability of obtaining a real bacterial enzyme with two hundred amino acids? The answer is given by multiplying the probability of each correct amino acids in the sequence, 1 out of 20, for 200 times, so as to produce quite a small probability: 1 out of 20^{200}. Moreover, given the fact that it is not sufficient to create a single enzyme to duplicate a bacterium, but it would rather be necessary to assemble about two hundred functioning enzymes, the probabilities would become 1 out of 10^{40000}, that is to say almost zero. If the total number of attempts made to create life is only 10^{51}, and the possibilities are 1 out of 10^{40000}, then the two scholars come to the conclusion that life would not have been able to develop. In that case, Monod would be right: we would really be a number which came up on a roulette, that is to say, we would be lucky and impossible. However, Kauffman supports a different idea. Hoyle and Wickramasinghe gave up the idea of spontaneous generation of life since the probability of a similar event occurring was comparable to the possibilities that a tornado, by passing over a rubbish dump, would be able to assemble a Boeing 747 with the materials deposited there. The two scientists, however, underestimated the power of self-organization. According to Kauffman, in fact, it

is not necessary that a specific group of enzymes is assembled, one after the other, to carry out a specific number of reactions:

There are compelling reasons to believe that whenever a collection of chemicals contains enough different kinds of molecules, a metabolism will crystallize from the broth. If this argument is correct, metabolic networks need not be built one component at a time; they can spring full-grown from a primordial soup. Order for free, I call it. If I am right, the motto of life is not We the improbable, but We the expected. (Kauffman, 1995, p. 45).

These words of the American chemist concerning free order, offer a version of the genial ideas, although intuitive ones, which animated the primitive Darwinian consciousness of complexity. This hypothesis of the origin of life, elaborated by the great scientist, constitutes therefore a radical answer to the disturbing questions posed by Monod in the final part of his volume. Are we really in the exceptional condition of the one who has just won a thousand million? Is life really that miraculous an event which has occurred casually and only once? And finally, since the genetic code can only be translated by the same products of the translation, when and how has such ring closed-in on itself? Most scientists are convinced that the initial life has had simple features and it became complex only later. Whereas, according to Kauffman, life is not bridled by the magic of bases and replications, yet it is based on a deeper logic. It is a natural property of the complex chemical systems: when the number of different molecular species in a chemical broth passes a certain threshold, a net of self-sustaining reactions – an autocatalytic metabolism – suddenly appears. If Kauffman's hypothesis is true it means that life has not appeared in a simple form, but complex and articulated, and it has remained complex and articulated ever since:

The secret of life, the wellspring of reproduction, is not to be found in the beauty of Watson-Crick pairing, but in the achievement of collective catalytic closure. The roots are deeper than the double helix and are based in chemistry itself. So, in another sense, life – complex, whole, emergent – is simple after all, a natural out-growth of the world in which we live (Kauffman, 1995, p. 48).

5. Towards the theory of complexity

An attentive theoretical and experimental work carried out by Kauffman for more than forty years, of which almost twenty were spent at the *Santa Fe Institute*, firmly supports the original theories just mentioned which, under some aspects, are also revolutionary ones. The referring theoretical fire of the great American scientist is represented by what chemistry calls catalysis. Catalysts, as for example enzymes, accelerate chemical reactions which otherwise could only proceed very slowly. In general, many chemical reactions proceed with difficulty: given a great expanse of time, in fact, some molecules of A might combine with B to make C, but, as we have recently pointed out, in the presence of a catalyst (a molecule D) the reaction proceeds way faster. So, as Kauffman says, if D is the catalyst combining A and B to make C, the molecules A, B and C might themselves act as catalysts for other reactions. That being the case, it is possible to define a living organism as that "system of chemicals substances which is able to catalyze its own reproduction". What Kauffman defines as " a collectively autocatalytic system" is something in which molecules speed up the very reactions by which they are formed, that is a system in which, for example, A makes B, B makes C and C makes A again. Now we may consider an entire network of these self-supplied circles; given a source of molecules as food, the network will

be able to recreate itself constantly. Such network, according to Kauffman, must be considered like the real metabolic networks residing in living cells, which means that, from a theoretical point of view, it is alive. The American scholar would like to demonstrate that if a group of sufficiently different molecules accumulate in one point, the possibilities of obtaining a self-supporting and self-reproducing metabolism (an autocatalytic system) becomes almost a certainty. According to this thesis the *bios* originates in the property of catalytic closure among different molecular species: if taken alone, in fact, each molecular species is inert, however, once the autocatalytic closure is obtained, the collective system of molecules comes to life.

[…] Every free-living cell, is collectively autocatalytic. No DNA molecules replicate nude in free-living organisms. DNA replicates only as part of a complex, collectively autocatalytic network of reactions and enzymes in cells. No RNA molecules replicate themselves. The cell is a whole, mysterious in its origins perhaps, but not mystical. Except for «food molecules», every molecular species of which a cell is constructed is created by catalysis of reactions, and the catalysis is itself carried out by catalysts created by the cell. To understand the origin of life, I claim, we must understand the conditions that enabled the first emergence of such autocatalytic molecular systems. (Kauffman, 1995, p. 50).

Catalysis alone is not enough to explain life; living systems are in fact open non- equilibrium thermodynamic systems because they exchange matter and energy to reproduce, unlike close thermodynamic systems which are chemical systems in equilibrium since they do not absorb neither matter nor energy from the environment. Usually chemical reactions can be more or less reversible: A transforms into B, but B transforms into A. So, by starting exclusively with molecules A, the concentration of B would increase to the point where the velocity of conversion of A into B would be exactly equal to the velocity of conversion of B into A. This balance is defined as chemical equilibrium: the concentration of A in B does not change with time, but every single molecule of A can transform into B and vice versa as far as thousands times a minute. As we have already mentioned, catalysts (protein enzymes and ribozymes) can accelerate in equal measure the reactions: the equilibrium between A and B is not altered because the enzymes simply increase the velocity to reach the state of balance. Between A and B there is an intermediate state defined as transition state in which some bonds among the atoms of the molecules are tightened and distorted: the molecule in the transition state is quite unhappy and the length of this unhappiness is given by the same energy of the molecule; low energy corresponds to slightly tense molecules, whereas high energy corresponds to tense molecules. This being the case, it is clear how enzymes function by linking and stabilizing the transition phase: they increase the speed to reach the equilibrium of the ratio of concentrations of A and B. For a living system chemical equilibrium equals death. The living beings are dynamic open systems which are constantly far from chemical equilibrium (Di Bernardo, 2010). Thus, such systems obey rules which are very different compared to those of closed systems: here the ratio between A and B will be reversed in comparison with the thermodynamic equilibrium ratio.

Let us consider now a much more complex open system: the living cell. If we are hoping that by understanding the behavior of very simple, open chemical thermodynamic systems we will be able to understand the cell we are really conceited. At present, nobody understands how the complex cellular networks of chemical reactions and their catalysts behave, or which laws govern their behaviors. Ilya Prigogine has called these systems "dissipative" because they continuously dissipate matter and energy to be able to preserve their structure. Unlike simple stationary systems in the thermodynamically open flask, the

concentrations of chemical species in a more complex dissipative system may not be able to attain a stationary state, unchanged through time. Concentrations instead, can begin to oscillate up and down in repeated cycles, called limit cycles, which last for long periods of time. Such systems can also generate spatial trends of a certain relevance. (Prigogine, 1967; Prigogine & Stengers, 1988, 1979; Glansdorff & Prigogine, 1971; Nicolis & Prigogine, 1977). For instance, the Belosov-Zabotinskij reaction, recalled several times by Kauffman, develops through some simple organic molecules and it generates two type of spatial structures: in the first one concentric waves stretch by propagating outside, whereas in the second one we have spirals rotating around two central points. The heart is an open system and it can pulse by following a trend similar to the Belosov-Zabotinskij reaction: a sudden death, in fact, caused by a cardiac arrhythmia could correspond to the passage from what is the corner of a concentric rings scheme (heartbeat regularity) to a spiraling scheme inside the myocardium. Even so, Kauffman strongly reaffirms that, although very interesting, such chemical systems are not yet living systems: the cell, in fact, is not only an open chemical system, but a collectively auto- catalytic one. In the cells we have not only the emergence of chemical trends, but cells maintain themselves as entities able to reproduce and head towards a Darwinian evolution. But which laws, which deep principles may have caused the emergence of autocatalytic systems on primordial earth? What we are looking for is the myth of our creation (Kauffman 1995, 2008).

6. Autocatalytic systems and computer simulation

Since the Eighties, Kauffman has dealt with this complex problem by working on more simple models made easily feasible through computer simulation. Thus, through a casual graph, that is a group of points or nodes which are connected in a casual way by a series of lines, the American scholar attempts to build an approximate model which could explain the secret behind autocatalytic systems. Let us consider, for example, 10000 buttons scattered on the floor; we then choose two buttons at random and we connect them through a thread; done with this couple, we choose two other buttons and, as for the previous couple, we connect them through a thread. Going on in fits with this operation, the more we pick up buttons the more the probability to pick up at random two buttons and find out that one of them has been already picked up increases: so after a while the buttons will begin to be connected in big groups. The group which is interconnected in the casual graph is called component; some buttons can appear as not connected to other buttons, whereas others can be connected as a pair, as groups of three or more. The important characteristics of casual graphs show a very regular statistical behavior when the ratio between threads and buttons is modified. In particular, a phase transition occurs when the ratio between threads and buttons exceeds the value of 0.5. At this point, a gigantic group suddenly forms (Kauffman, 1993, 1995). In the 10000 buttons model the gigantic component emerges in the presence of 5000 threads. When such component forms, most nodes are directly or indirectly connected. As the ratio of the threads compared to the buttons keeps increasing above the threshold of 0.5, more and more buttons and small isolated groups will connect with the gigantic component. In this way, the gigantic structure expands itself, but the pace of growth decreases in accordance with the decreasing of the number of components that are not yet isolated. The quite sudden change of dimensions of the bigger group of buttons, when the ratio between threads and buttons exceeds 0.5, according to Kauffman, is a toy version of the phase transition which could have led to the origin of life. If there were an infinite

number of buttons, when the ratio between threads and buttons should exceed 0.5 the dimensions of the bigger components would hope discontinuously from the minuscule to the enormous (Langton, 1990). This is a phase transition, like separated water molecules freezing in a single block of ice. Kauffman's thesis is therefore simple: with the increase of the ratio between threads and buttons, suddenly so many buttons become connected that an enormous network is formed in the system. This gigantic component is not a mystery: its formation is the expected natural property of a casual graph (Figure 1). The analog of the origin of life is that when a sufficiently elevated number of reactions are catalyzed in a system of chemical reactions, an enormous network of catalyzed reactions will crystalize immediately. And, as it is, such a network will almost surely be autocatalytic, able to self-sustain and alive (Kauffman, 1995). In the attempt to understand the origin of collectively autocatalytic molecular systems, Kauffman draws some graphs of metabolic reactions in which circles represent chemical substances and squares represent reactions (Figure 2). Besides, he distinguishes the spontaneous reactions which should occur very slowly from the catalyzed ones which, on the contrary, should occur quickly (Figure 3). The goal is to find the conditions in which the same molecules are both the catalysts and the products of the reactions generating the autocatalytic group. This depends on the possibility for each molecule of the system to have a double function: it can act as an ingredient or as a product of a reaction, but it can be the catalyst of another reaction too (Kauffman, 1995). RNA proteins and RNA molecules have this double function: any type of organic molecule, in fact, can be both the substrate and the product of reactions and, at the same time, can act as a catalyst to speed up other reactions. According to Kauffman, if one would know with certainty which group of molecules catalyze certain reactions, it would be possible to foresee

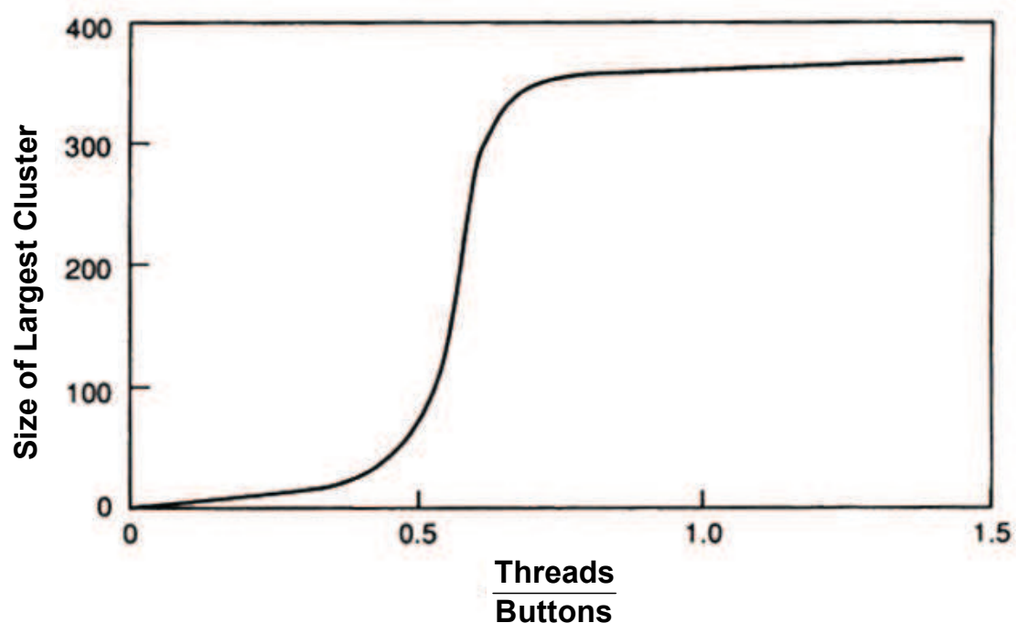

Fig. 1. A phase transition. As the ratio of threads (edges) to buttons (nodes) in a random graph passes 0.5, the size of the connected cluster slowly increases until it reaches a "phase transition" and a giant component crystallizes. For this experiment, the number of threads ranges from 0 to 600, while the number of buttons is fixed at 400 (Kauffman, 1995, p. 57).

with precision which group of molecules could be collectively autocatalytic. Although this knowledge is not available, it is anyway possible to proceed with an approximation by formulating plausible hypotheses and this is because "the spontaneous emergence of self-sustaining webs is so natural and robust that it is even deeper than the specific chemistry that happens to exist on earth; it is rooted in mathematics itself " (Kauffman, 1995, p. 60). Thus, this being the case, it becomes natural to wonder on the probability that a similar self-sustained network of reactions would form naturally. That is to say, is the appearance of collective autocatalytic reactions probable or virtually impossible? Kauffman answers as follows:

The emergence of autocatalytic sets is almost inevitable. […]As the ratio of reactions to chemicals increases, the number of reactions that are catalyzed by the molecules in the system increases. When the number of catalyzed reactions is about equal to the number of chemical dots, a giant catalyzed reaction web forms, and a collectively autocatalytic system snaps into existence. A living metabolism crystallizes. Life emerges as a phase transition. (Kauffman, 1995, pp. 61-62).

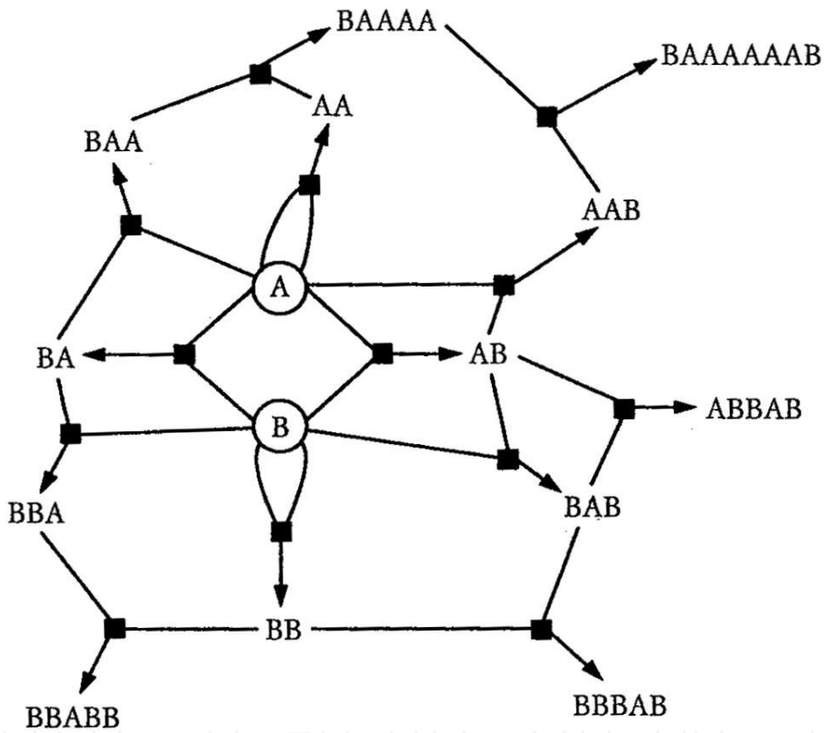

Fig. 2. From buttons and threads to chemicals. In this hypothetical network of chemical reactions, called a reaction graph, smaller molecules (*A* and *B*) are combined to form larger molecules (*AA, AB, etc.*), which are combined to form still larger molecules (*BAB, BBA, BABB, etc.*). Simultaneously, these longer molecules are broken down into simple substrates again. For each reaction, a line leads from the two substrates to a square denoting the reaction; an arrow leads from the reaction square to the product. (Since reactions are reversible, the use of arrows is meant to distinguish substrates from products in only one direction of the chemical flow.) Since the products of some reactions are substrates of further reactions, the result is a web of interlinked reactions (Kauffman, 1995, p. 59).

With the increasing of the diversity and complexity of the molecules in the system also the ratio between reactions and chemical substances in the reaction graph increases: there are, in

fact, more reactions through which molecules can be formed than molecules. What happens, therefore, to the ratio between reactions and molecules in the graph when both the complexity and the diversity of such molecules increase? After some short algebraic calculations, it is easy for simple linear polymers to demonstrate that by increasing the length of the molecules the number of molecular species increases exponentially, but the number of reactions through which they transform themselves from one to the other increases even faster. Thus, the chemical system becomes more and more a fertile source of reactions which constantly transform themselves into other molecules. However, in order for the system to generate autocatalytic networks which are able to self-sustain, some of the molecules must act as catalysts by speeding up the reactions. For now, in fact, the system is fertile, but not yet a carrier of life since we do not know with certainty which molecule catalyzes which reaction. Among the different models built by Kauffman the most simple and functional model is the one supposing that each polymer has prearranged possibilities, one out of a million, to be able to function as an enzyme to catalyze a certain reaction. By using this model we decide beforehand which reactions each polymer is able to catalyze. This rule is defined by the American chemist as the "random catalysis rule" and it consists of assigning, at random and in a definite way, to each polymer the reactions it is able to catalyze. In this way, we will be able to distinguish the catalyzed reactions, trace some arrows from the catalysts to the reactions they catalyze and finally wonder if such a model

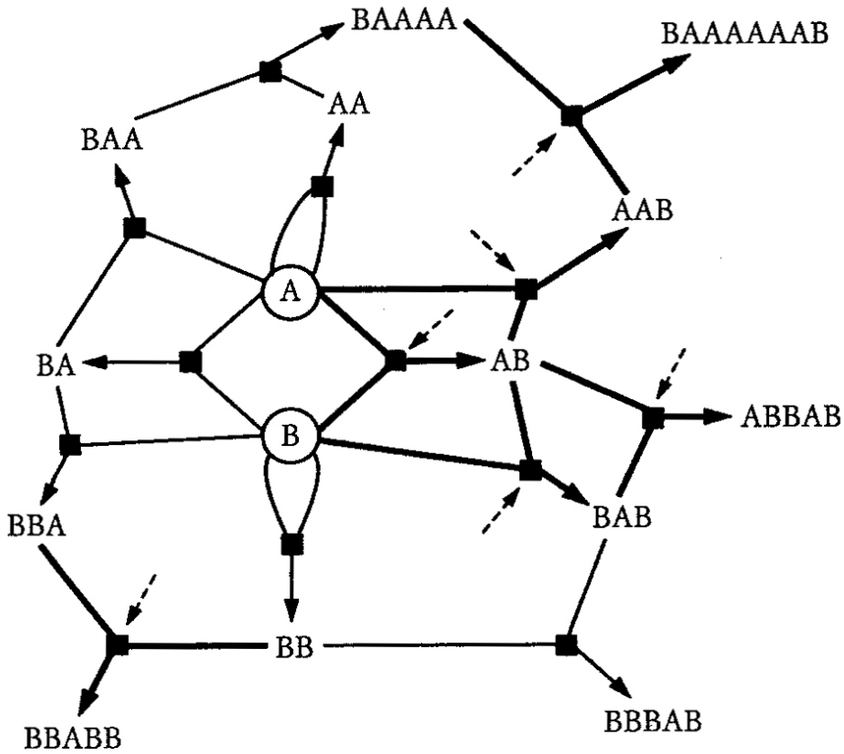

Fig. 3. Molecules catalyzing reactions. In figure 2, all the reactions were assumed to be spontaneous. What happens when we add catalysts to speed some of the reactions? Here the reaction squares indicated by dashed-line arrows are catalyzed, and the heavy, darker lines connect substrates and products whose reactions are catalyzed. The result is a pattern of heavy lines indicating a catalyzed subgraph of the reaction graph (Kauffman, 1995, p. 61).

of chemical system contains a collectively autocatalytic group, that is a network of molecules connected through thick lines also containing the molecules catalyzing the reactions by which the same molecules are formed. In order to make the model more realistic, Kauffman adds a second rule: even if a candidate to the role of ribozyme has a site which fits the right and left end of its substrates, it has only a probability out of a million to have other chemical properties which can make it catalyze the reaction. This suggests the idea that also other chemical characteristics could be necessary, besides the complementarity of the bases, to obtain a catalysis made by the ribozyme. The American biologist calls this "complementary catalysis rule". And here it is the crucial result: it does not matter which of these catalyst rules are used; when a group of model rules reaches a critical diversity, a gigantic component of catalyzed reactions crystalizes, causing the emergence of collectively autocatalytic groups. At this point it is not difficult to understand the reason why this emergence is "virtually inevitable" (Kauffman, 1995). Supposing we establish that any polymer has a possibility out of a million to be able to act as an enzyme for a certain reaction and that we have to use the random catalyst rule, it means that with the increasing of

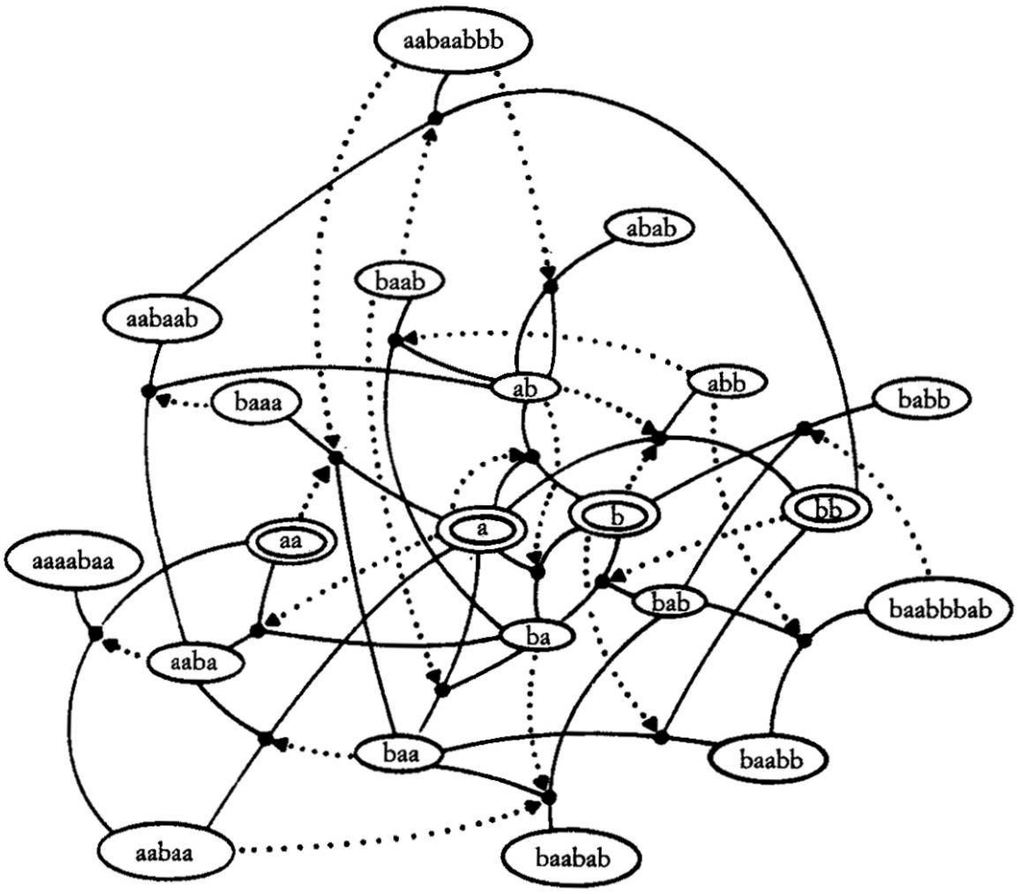

Fig. 4. An autocatalytic set. A typical example of a small autocatalytic set in which food molecules (*a*, *b*, *aa*, *bb*) are built up into a self-sustaining network of molecules. The reactions are represented by points connecting larger polymers to their breakdown products. Dotted lines indicate catalysis and point from the catalyst to the reaction being catalyzed (Kauffman, 1995, p. 65).

diversity among the molecules of the model system, the ratio between reactions and molecules will increase. When the diversity of the molecules is quite elevated, Kauffman says, the ratio between the reactions and the polymers reaches the level of a million to one. At this level of complexity then, each polymer will be able to catalyze a reaction: when the ratio between catalyzed reactions and chemical reactions is 1, there is an extremely high probability for a gigantic component, a group of collectively autocatalytic molecules, to form. In the inert broth, for example, nothing happens, apart from very slow spontaneous chemical reactions. If both the diversity and the atomic complexity of molecules are increased, more and more often the same elements of the system will catalyze internal reactions (Figure 4). When a certain threshold of diversity is exceeded, an enormous network of catalyzed reactions arises during the phase transition: "the giant component will contain a collectively autocatalytic subset able to form itself by catalyzed reactions from a supply of food molecules" (Kauffman, 1995, p. 64).

7. Conclusion

Thus, the theory about the origin of life, as elaborated by Kauffman[3], has its roots in an irreducible holism which is not the result of reflections of a metaphysical nature, but of mathematical necessity: life has emerged as whole, not a little at a time, and it has remained that way. Therefore, unlike the dominant vision which sees the naked RNA at the origin of life, with its customized evolutionary stories, today we have the hope of explaining why living creatures seem to have a minimal complexity, because nothing simpler than a *pleuromona* can be alive (Kauffman, 1995). However, if life has begun by collective autocatalysis and has incorporated the DNA and the genetic code only later, how can catalytic groups meet with hereditary variations and with a natural selection without yet containing a genome? In other words, is there a way for an autocatalytic group to evolve without all the complications related to the genome?

Richard and Doyne found a natural way for the variation and evolution in such systems to develop. They suggested that a casual and not catalyzed reaction can develop occasionally when an autocatalytic network is active from a metabolic point of view. Such spontaneous fluctuations will tend to generate molecules which are not part of the group; these new molecules, according to the two scholars, can be considered as a sort of "half-light of molecular species" and as a "chemical haze surrounding the autocatalytic group". The original group, by absorbing some of these new molecular species on the inside, would be altered. At this point two cases are possible: A) if one of these new molecules contributed to catalyze its own formation, it would became a member of the network to all intents and purposes and so, a new circuit would be added to the metabolism; B) if the molecular intruder inhibited an already existent reaction, then an old circuit could be eliminated from the group. In both cases a hereditary variation is clearly possible. Moreover, if the result would be a more efficient network, such mutations would be favored and the modified network would take the place of weaker competitors. With reference to such considerations, Kauffman sustains that there are several reasons to believe that autocatalytic groups could evolve even without a genome. Biologists divide cells and organisms by genotype (genetic

[3] We must take into consideration that the autocatalysis theory has been recently revisited by Kauffman in 2000, in 2007, and in 2008. For a thorough examination informations of the possible limits of the emergence theory elaborated by the American scholar consult: Carsetti, 2009; Di Bernardo, 2011.

information) and phenotype (enzymes and other proteins, but also organs and morphological characteristics constituting the body). For autocatalytic groups there is no separation between genotype and phenotype. The system acts as its own genome. Nevertheless the capability to incorporate new molecular species, and perhaps eliminate older molecular forms, gives us the promise to generate a network of self-reproducing chemical substances equipped with different characteristics. Darwin teaches us that such systems will evolve by natural selection (Kauffman, 1995). It is inevitable that these proto-cells which are self-reproducing and divided up together with their "daughters", will form a complex ecosystem. It is not metabolic life which has begun as a complex whole but, according to the biochemist, the entire panoply of mutualism and competition we define as ecosystem is blossomed from the beginnings. The story of these ecosystems at all levels is not only the story of evolution, but also of coevolution. These considerations give reasons of the Darwinian intuition concerning the internal constitution of complexity (Darwin, 1902). From what has been said since this moment, in fact, it is clear how, in Kauffman's opinions, autocatalytic systems are not chaotic at all. If life has begun when some molecules spontaneously united to form autocatalytic metabolisms, we will then find a source of molecular order, a fundamental source of internal homeostasis which protects cells from perturbations, and a compromise which let the proto-cellular networks receive light fluctuations without collapsing (Kauffman, 2004a, 2004b). This order that without the genome emerges from the collective dynamics of the network and from the coordinated behavior of associated molecules, as we have abundantly demonstrated through Kauffman's words, is free and is generated by small attractors which, under some conditions, represent the source of order of big dynamic systems. Thus, in this context the concept of catalytic closure in groups of collectively autocatalytic molecules appears as a profound model of the complexity laws, that is those powerful laws which, even if they are not able to supply a detailed prediction of the "three of life" they are able to explain its general form. Life, from this point of view, appears as an emerging phenomenon which develops when the molecular diversity of a pre-biotic chemical system exceeds a given level of complexity. If so much is true, then life does not reside in the individual properties of each single molecule- in details- but it is a collective property of systems of molecules interacting with each other. From this perspective, life has emerged as a whole and has always remained so. From this point of view, life must not be searched in its parts, but in the totality of the emerging properties creating the whole. A group of molecules can have or not the capability of catalyzing its own formation and reproduction starting from simple basic molecules. In the emerging and self-reproducing whole there is no vital force or foreign substance. And anyhow, "the collective system does possess a stunning property not possessed by any of its parts. It is able to reproduce itself and to evolve. The collective system is alive. Its parts are just chemicals" (Kauffman, 1995, p. 24). If these ideas are right, it means that, we are not only "at home in the universe", but we actually have excellent probabilities to share it with some other being which is still unknown (Kauffman, 2001).

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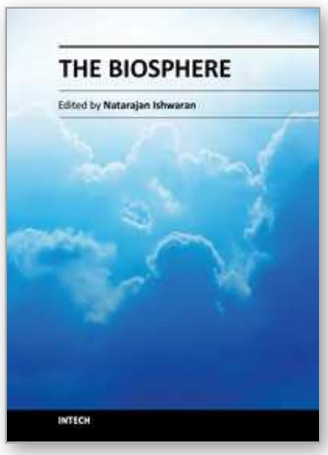

The Biosphere

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In this book entitled "The Biosphere", researchers from all regions of the world report on their findings to explore the origins, evolution, ecosystems and resource utilization patterns of the biosphere. Some describe the complexities and challenges that humanity faces in its efforts to experiment and establish a new partnership with nature in places designated as biosphere reserves by UNESCO under its Man and the Biosphere (MAB) Programme. At the dawn of the 21st century humanity is ever more aware and conscious of the adverse consequences that it has brought upon global climate change and biodiversity loss. We are at a critical moment of reflection and action to work out a new compact with the biosphere that sustains our own wellbeing and that of our planetary companions. This book is a modest attempt to enrich and enable that special moment and its march ahead in human history.

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